

The South African Journal *of* **Medical Laboratory Technology**

ORGAN OF THE SOCIETY OF MEDICAL LABORATORY
TECHNOLOGISTS OF SOUTH AFRICA

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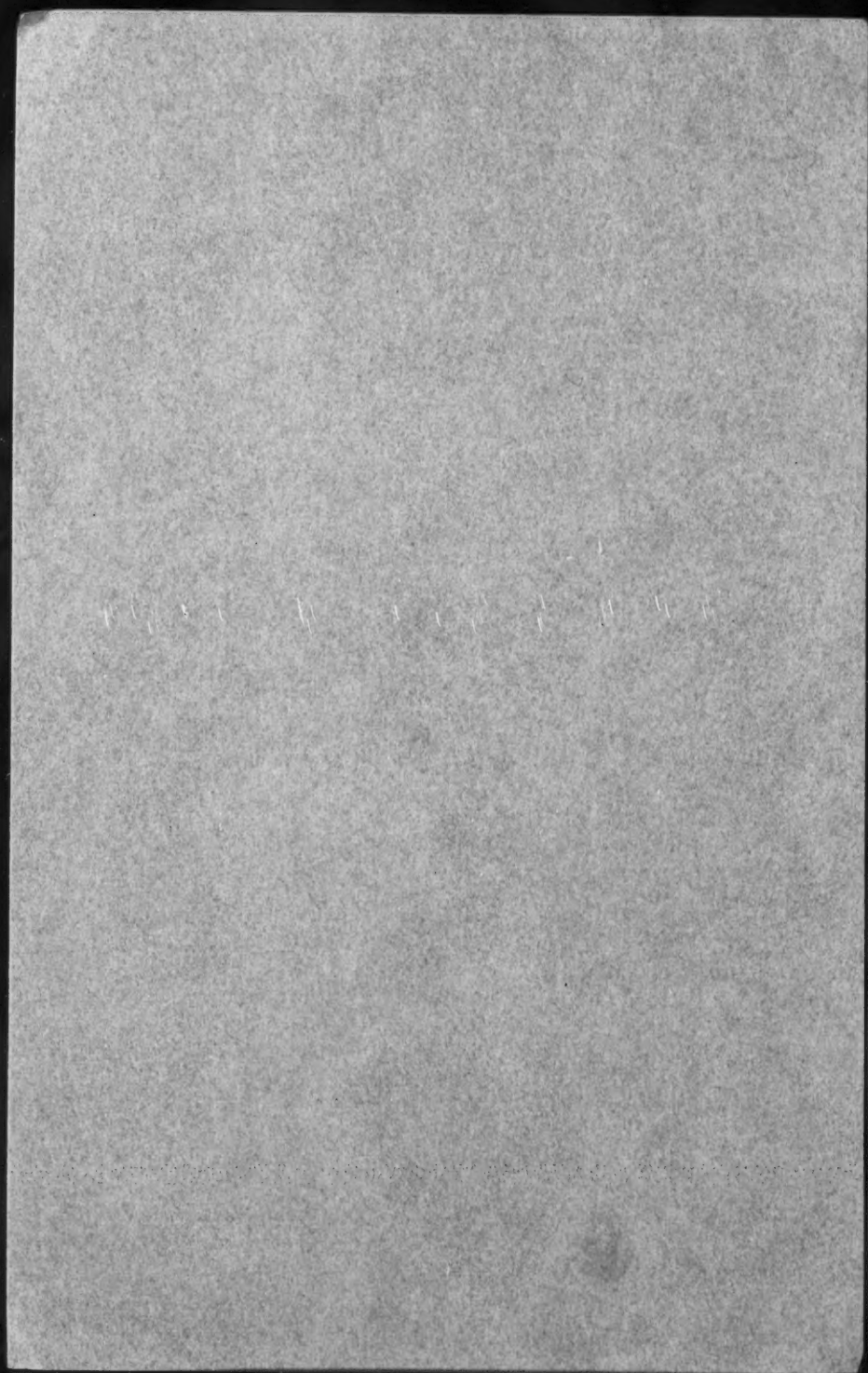
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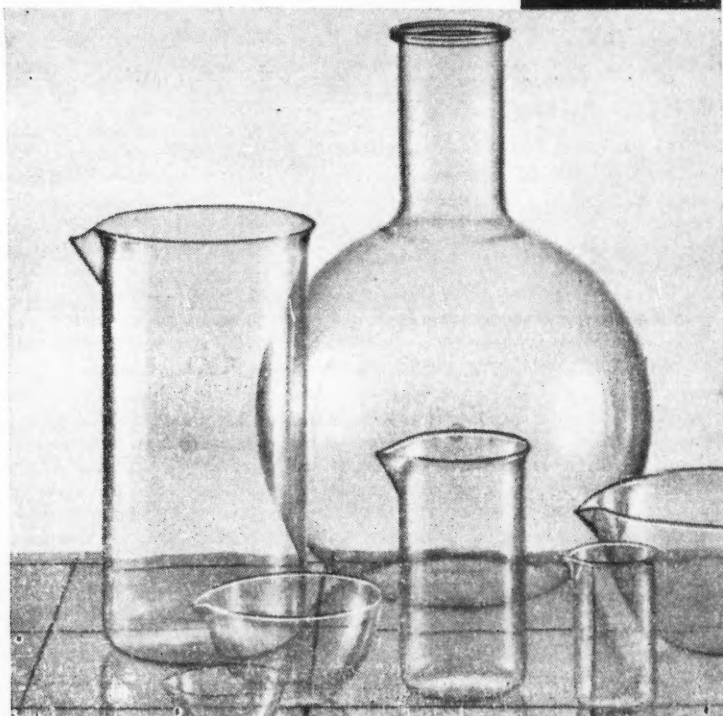
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THE ISOLATION AND IDENTIFICATION OF CLOSTRIDIA

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Department of Pathology, University of Natal, Durban

The isolation and identification of spore-bearing anaerobes from mixed cultures presents the technician with certain difficulties. Experience gained in overcoming some of these difficulties is recorded. However, this article does not pretend to be exhaustive.

MATERIALS AND METHODS

The isolation and identification of *Cl. tetani*

Isolation: Specimens from the umbilici of clinical cases of tetanus neonatorum and samples of kraal manure were examined. The method finally adopted is as follows:

Pieces of umbilical tissue were macerated in a Griffith's tissue-grinding tube with the aid of about 1 gm. sterile builder's sand and 2 mls. sterile broth. The mixture was then decanted into two tubes of Robertson's Cooked Meat Medium (R.C.M.M.). Swabs of umbilical exudate and samples of kraal manure were inoculated into 2 tubes of R.C.M.M. One tube of R.C.M.M. in each instance was heated at 60°C. for 30 mins. All tubes of R.C.M.M. were then incubated for 7 days to allow *Cl. tetani* organisms present to grow and form spores.¹ Films of the cultures were then made, stained by Gram's, and examined for the presence of Gram positive bacilli with round terminal spores. If no organisms resembling the tetanus bacillus were observed the tubes were incubated for a further 7 days and re-examined. On finding organisms morphologically resembling *Cl. tetani*, 0.5 ml. of each culture was distributed into a sterile 5" x $\frac{5}{8}$ " tube and placed in boiling water. At intervals of five minutes a loopful from each tube was inoculated into a tube of semi-solid glucose agar medium.² The cultures were boiled for a total of 20 minutes. The inoculated semi-solid glucose agar cultures were incubated overnight and then examined for the amount of growth and gas production. Heavy growth and much gas production is evidence of growth other than *Cl. tetani*, although *Cl. tetani* may also be present. Tubes showing weak or no growth were re-incubated and examined at intervals up to four days for the typical type of tetanus growth in this medium which is slightly opalescent and rather streaky to begin with, the growth being present up to $\frac{1}{2}$ " from the surface of the medium.

Identification: Subcultures were made from tubes likely to contain a pure growth of *Cl. tetani* on to blood agar plates, and duplicate plates were incubated aerobically and anaerobically for 18 hrs. The plates were examined for the pure anaerobic growth of an organism giving the typical "feathery" type of growth of *Cl. tetani*. Films of such growths were

stained for purity and if pure were subcultured into R.C.M.M. and again incubated and examined for sporulation and purity. From R.C.M.M. cultures having pure growths of Gram positive bacilli with round, terminal spores, biochemical tests were set up as described by Spray,² and mice were inoculated. Twelve mice were given 0.1 ml. culture in R.C.M.M. and 0.1 ml. 10% lactic acid intramuscularly³. Six of the mice were then given 1,500 units tetanus antitoxin intraperitoneally. In cases where all the unprotected mice died showing signs of typical tetanus and all the mice which received antitoxin lived, the organism was reported as *Cl. tetani*. In these cases the biochemical tests gave the results expected of *Cl. tetani*.

Discussion: Using this method the tetanus bacillus was isolated and identified in four cases. It should be noted that in each of these cases pieces of tissue were submitted for examination. According to Stokes⁴, the chances of isolating pathogens are much higher when tissue is examined than when exudate is examined. Altogether, since this method was adopted, 17 specimens from umbilici were examined, but at least half of this number were not pieces of tissue. The results obtained by the above technique compare favourably with those obtained by Hauduray and Rosset⁵ who examined 13 pieces of wound tissue from cases of clinical tetanus and isolated the bacillus five times.

In the glucose semi-solid agar cultures examined, it was evident that tetanus spores are very resistant to boiling. Usually boiling after 5 minutes resulted in heavily contaminated cultures; after 10 minutes occasionally one tube revealed a pure growth of *Cl. tetani*; after 15 minutes one or more pure growths of *Cl. tetani*; and after 20 minutes almost pure growths of the tetanus bacillus in all tubes. However, after the original culture had been boiled for 20 minutes some of the subcultures showed no growth. These observations concur with those of Wilson & Miles¹ where it is stated that tetanus spores resist boiling from 15 to 90 minutes.

A few strains of *Clostridia* which were morphologically, biochemically and culturally identical to *Cl. tetani* have been isolated, especially from the kraal manure samples. However, these strains were not lethal to mice. According to Spray² such organisms should be labelled *Cl. lentroputrescens*; however Wilson & Miles¹ state that approximately half the number of strains of tetanus isolated from animal faeces proved to be non-toxic.

Other methods such as blood agar slope method,⁶ use of chloral hydrate,³ use of sodium azide,⁷ decimal dilutions in agar,⁶ were tried but all failed to render pure cultures of a Gram positive bacillus with terminal spores so often seen in films of R.C.M.M. cultures mixed with numerous other types of *Clostridia*.

The Isolation and Identification of *Cl. welchii*

Isolation: Specimens of faeces and pus were planted on to sodium azide blood agar plates⁷ and incubated aerobically and anaerobically.

R.C.M.M. tubes were also inoculated and in the case of pus a film was made and stained by Grams.

Ward dust samples were placed in R.C.M.M. All cultures were incubated for 18 hrs., the plates examined for typical *Cl. welchii* colonies and the R.C.M.M. plated out, incubated as above and examined for typical *Cl. welchii* colonies.

Identification: Typical *Cl. welchii* colonies were stained by Gram's and if organisms morphologically resembled *Cl. welchii* they were tested for fermentation of lactose and neutralisation of lecithinase activity on an egg plate which was prepared as follows.

The yolk of a fresh egg was separated, aseptically, into a sterile petri dish and mixed with an equal volume (usually about 15 mls.) of sterile 0.85% saline. 90 mls. of sterile nutrient agar containing 0.003% neutral red was melted and cooled to 50°C. To this was added 10 mls. 10% lactose and 10 mls. egg-saline mixture. The media was well mixed and poured in the usual way. As many plates as required were dried and on one half *Cl. welchii* type A antitoxin was spread, the plates dried again and streaked with the suspected *Cl. welchii* colonies. Plate I demonstrates the results obtained with *Cl. welchii* organisms. The neutralisation

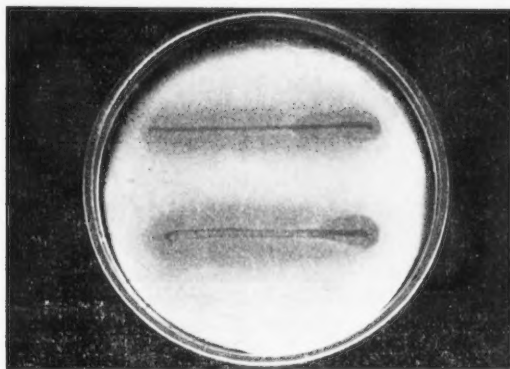


Plate I.—Egg agar plate inoculated with two strains of *Cl. welchii*. *Cl. welchii* antitoxin was smeared on the left half of the plate. Photographed after 18 hrs. anaerobic incubation.

of the lecithinase activity is seen by the absence of the heavy precipitate around the culture on the side treated with the antitoxin. The fermentation of lactose with the production of acid is seen as a red halo surrounding the culture—in Plate I this appears as deep grey diffusing from the culture streak. The method above is condensed from McClung & Toabe⁸ and Willis & Hobbs.⁹

As *Cl. welchii* is the only *Clostridium* to ferment lactose and produce lecithinase which is neutralised by the *Cl. welchii* antitoxin, the identification is complete.

This method was checked biochemically and on serum and Fildes extract plates,⁶ and no discrepancies were observed.

Discussion: The egg plate, without antitoxin, may be used for primary isolation of *Cl. welchii*, Plate II, where *Cl. welchii* colonies are identified by the precipitation surrounding them, but in specimens where spreading bacteria such as *B. proteus* are likely to occur the sodium azide blood agar plates proved more satisfactory. The egg plate, with one half

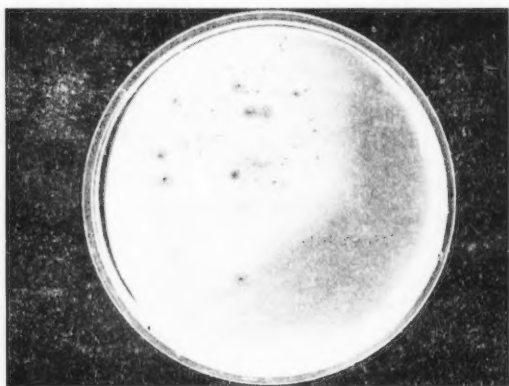


Plate II.—Egg agar plate inoculated with a specimen of pus from a case of gas gangrene. Photographed after 18 hrs. anaerobic incubation.

treated with antitoxin, may be used as a rapid method of identifying *Cl. welchii* in specimens where this organism is predominating; it has not been tried in this way here. In Plate II the predominating organism was *B. coli* hence the evidence of lactose fermentation around the initial inoculum.

The advantages of the egg plate over the medium containing human serum and Fildes extract⁶ are:—

- (a) The egg plates are more easily prepared. No source of sterile human serum is needed. Provided a sufficiently nutritious agar base is used⁸ no additive such as Fildes extract, which takes a lot of time to prepare, is needed.
- (b) The use of egg as a source of lecithin gives more consistent results and is more sensitive.¹⁰

It should be noted too that (a) the egg plates keep when refrigerated for at least 8 weeks, and (b) even without the lactose and neutral red they are very satisfactory for the demonstration of neutralisation of lecithinase by the specific antitoxin.

Identification of other species of Clostridia

The biochemical activities of the genus are important in identification but are usually fraught with difficulties. The sugar tubes, etc. take up too much room in the anaerobic jar; indicators are reduced under anaerobic conditions; if iron is used as a reducing agent the results are often masked by discoloration of the medium. The use of semi-solid media as described by Spray² precludes many of these difficulties. Most of the ingredients for the media are readily obtainable and the media are easily made up.

The egg plate had been stated to be useful in identifying a number of the more common Clostridia by examining the type of growth, surface of the growth, action on the egg, etc. by McClung and Toabe⁸ and Willis & Hobbs.¹¹ However, it is thought that considerable experience is necessary before one would be justified in relying entirely on this way of identifying the species of Clostridia mentioned in the articles.

SUMMARY

1. A method for isolating *Cl. tetani* from cultures of mixed Clostridia is described.
2. A simple method for isolating and identifying *Cl. welchii* is described.
3. Reference is made to means of identifying other Clostridia.

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ACKNOWLEDGMENTS

I wish to thank Professor J. Wainwright and Dr. K. C. Watson for permission to publish these findings; Mr. C. R. Stuart for taking the photographs and Mrs. S. E. Maddison for her encouragement and many helpful suggestions.

CURRENT AFFAIRS

Two topics are the concern of technologists in South Africa at the moment and both require an adequate airing as they are, respectively, of the utmost importance to us all. Firstly, in this issue we publish the text of the amended regulations, "Rules for the Registration of Medical Technologists". The substitution of these rules for those which have operated in the past represents a major advance in the profession of medical technology in the Union.

It is perhaps unfortunate that a little obscurity has crept into them and given rise to some difficulty. Note 8 to Rule 2 is a case in point and the trouble here appears to be in the interpretation of the word "any" in the phrase "any of the categories listed in rule 2 (c) (ii)". "Any" in this context appears to mean "one" and it follows, if this argument is carried to its logical conclusion that an applicant for exemption from the Intermediate Examination who intends to make say Haematology his choice for final registration may seek exemption from the Intermediate only if he has worked in a Haematology Laboratory for a two-year period. Admirable as this may seem to some, it defeats the purpose for which the Intermediate Examination was designed, which was to ensure that the candidate who wished to profess a subject other than "Clinical Pathology" should have a working, basic knowledge of general routine laboratory procedure.

We feel that the strict interpretation of this rule, which would allow a candidate to become registered as a medical technologist whilst having little or no knowledge of general laboratory procedure, to be faulty and such interpretation would, in fact, be a great disservice to medical technology as a whole.

We hope that the National Controlling Committee which is being formed will bear this fact in mind when it commences its deliberations later this year.

The second point of concern to us is the establishment of a "Diploma in Clinical Chemistry" course for Pharmacists at the School of Pharmacy, Natal Technical College, Durban. We have in the past jibbed at the use of the word "chemist" as a synonym for Pharmacist or Dispenser and may now perhaps be forgiven our feeling of alarm at the inclusion of many facets of clinical pathology in the syllabus for this course. Blood sugars, ureas, electrolytes, etc., blood counts including differentials, sedimentation rates, the examination of urine and faeces for parasites are included in this list of investigations which, it would seem, will shortly be available over the counter in your local "chemist's" shop. It is true that today the pharmacy is a glorified general store or bazaar but we had always understood that the pharmacist was concerned in the treatment of disease; now it appears that he wishes to diagnose too!

Food for thought as medical technology moves forward with the introduction of new rules.

GOVERNMENT NOTICE R.359/60

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DEPARTMENT OF HEALTH

No. R.359]

[18 March 1960.

THE SOUTH AFRICAN MEDICAL AND DENTAL COUNCIL**RULES FOR THE REGISTRATION OF MEDICAL
TECHNOLOGISTS**

The Minister of Health, in exercise of the powers conferred on him by sub-section (4) of section *ninety-four* of the Medical, Dental and Pharmacy Act, 1928 (Act No. 13 of 1928), as amended, has approved of the substitution of the following rules made by the South African Medical and Dental Council under section *ninety-four* read with section *thirty-two* of the said Act, for the rules published under Government Notice No. 2043 of 1949, as amended by Government Notices Nos. 668 of 1951 and 1397 of 1954:—

1. In these rules "the Act" means the Medical Dental and Pharmacy Act, 1928 (Act No. 13 of 1928), as amended, and any expression to which a meaning has been ascribed in the Act shall, when used in these rules bear the same meaning.

2. The Council may register as a medical technologist in one or more of the following categories, namely:—

Micro-biology,
Chemical Pathology,
Histopathology,
Haematology,
Blood Transfusion Technology,
Virology,
Parasitology and Helminthology,
Clinical Pathology,

any person who satisfies the Council that he has complied with the requirements set out in paragraphs (a), (b) and (c) of this rule: Provided that every person who, on promulgation of these rules, has been registered with the Council as a medical technologist under the provisions of Government Notice No. 2043 of 30th September, 1949, as amended, shall be deemed to be registered as a medical technologist in the category Clinical Pathology:—

- (a) That he holds an approved degree or diploma in Science of a recognised university or of an examining body approved by the Council from time to time as competent to grant such qualification;
- (b) that he has been trained for a period of at least four years in a laboratory or other institution approved of by the Council;

(c) that he has passed—

(i) an Intermediate Examination which shall comprise the following subjects:—

Chemistry,
Physics,
Anatomy,
Physiology,
Use of Instruments,
General Laboratory Technique;

(ii) a Final Examination to be taken—only after having trained for not less than three years in a laboratory or other institution approved for the purpose by the Council—in one of the following categories:—

Micro-biology,
Chemical Pathology,
Histopathology,
Haematology,
Blood Transfusion Technology,
Virology,
Parasitology and Helminthology,
Clinical Pathology:

Provided that a person who professes Clinical Pathology shall reach a satisfactory standard in each of the following subjects:—

Micro-biology,
Chemical Pathology,
Haematology.

NOTES TO RULE 2.

Notes (1)—The degree or diploma referred to in rule 2 (a) shall have included in its curriculum subjects which are substantially equivalent in content to the subjects prescribed in rules 2 (c) (i) and 2 (c) (ii) and the standard of teaching of these subjects and the examination in them shall be such as satisfy the Council. In so far as the Council is not satisfied that the subjects for the degree or diploma or the standard attained in such subjects comply with its requirements it may demand re-examination or further examination in any one or more such subjects.

Note (2).—A person holding a degree or diploma such as defined in rule 2 (a) may, at the discretion of the Council, be exempted or partially exempted from the examinations prescribed in rules 2 (c) (i) and 2(c) (ii); full exemption from these examinations by virtue of the provisions of this note will only be considered in the case of a person who has undergone academic training for a period of at least four years.

Note (3).—A person holding a degree or diploma such as defined in rule 2 (a) may, at the discretion of the Council, be exempted or partially

exempted from the training defined in rule 2 (b); full exemption from the training by virtue of the provisions of this note will only be considered in the case of a person who has undergone academic training for a period of at least four years; a person who holds an approved degree or diploma for which the curriculum extended over a period of not more than three years will, in addition to any examinations required under rule 2 (c), be required to undergo, subsequent to having obtained the approved degree or diploma, training such as is prescribed in rule 2 (b) for at least two years.

Note (4).—A person who is the holder of the matriculation certificate of the Joint Matriculation Board, or a certificate of exemption from the Matriculation Examination issued by the Board, or is the holder of a School Leaving Certificate, in the examinations for which the applicant obtained a pass in one of the subjects, Physical Science, Chemistry, Botany, Hygiene and Physiology, Physics, Biology, Zoology, or who subsequently passed an examination in one of these subjects equivalent to, or higher than the above examinations and who in addition has complied with the requirements prescribed in rules 2 (b) and 2 (c) above, may be registered.

Note (5).—A person who has been working as a medical technologist in a laboratory or institution approved by the Council in any of the categories listed in rule 2 (c) (ii) above for a period of five years prior to the 30th September, 1949, may, on the written recommendation of the head of that laboratory or institution, be exempted at the discretion of the Council from the examinations prescribed in rule 2 (c).

Note (6).—A person who has been working as a medical technologist in a laboratory or institution approved by the Council in any of the categories listed in rule 2 (c) (ii) above, for a total period of four years prior to the promulgation of these amended rules, may take a special examination in the subject or subjects of that category, which examination shall be designed primarily to test the candidate's practical knowledge of the subject he professes, and which shall be conducted by the Council and if successful, he may be registered; a person who is unsuccessful may be afforded one further opportunity to pass such a special examination; thereafter a person who desires registration shall have to comply with all the requirements of these rules: Provided that such a person may, at the discretion of the Council be exempted, or partially exempted, from the requirements of training in terms of rule 2 (b) above.

Note (7).—A person who has been working for a total period of less than four years prior to the promulgation of these amended rules as a medical technologist in a laboratory or institution approved by the Council in any of the categories listed in rule 2 (c) (ii) above, may, at the discretion of the Council, be granted a proportionate exemption from the requirement of training in terms of rule 2 (b) above.

Note (8).—A person who has been working for a total period of two years or more, prior to the promulgation of these rules as a medical

technologist in a laboratory or institution approved by the Council in any of the categories listed in rule 2 (c) (ii) above, may, at the discretion of the Council, be exempted from the requirement of passing an Intermediate Examination prescribed in terms of rule 2 (c) (i) above.

Note (9).—A person qualified for registration in one or more of the categories listed in rule 2 (c) (ii) above, shall be registered on the register of medical technologists, and the category or categories in which he is qualified shall be indicated after his name on the said register, and on the certificate of qualification issued to him.

3. Where, in the case of an application for registration, the institution or examining body on whose certificate of qualification the application is based, has not already been approved by the Council, the applicant shall be required to furnish the Council with authoritative information as to the standard of training given thereat, whereupon, if such standard of training is considered satisfactory by the Council, such institution or examining body shall be approved.

4. An applicant for registration under these rules shall be required to submit the evidence and qualifications by virtue of which he claims to be registered, together with—

- (a) a declaration of identity sworn before a justice of the peace or commissioner of oaths;
- (b) a certificate of good character signed by a registered person, a minister of religion, magistrate or other responsible person;
- (c) a certificate from a registered medical practitioner to the effect that the health of the applicant is not such as to render it inadvisable that such applicant should engage in his calling;
- (d) a sworn declaration before a justice of the peace or commissioner of oaths by the applicant that he has never been debarred from practice in any country by reason of misdemeanour or professional misconduct;
- (e) a fee of £5 for registration;
- (f) a birth certificate; or, if the applicant is unable to furnish a birth certificate, a baptismal certificate or other satisfactory evidence that he has attained the age of twenty-one years.

5. The Council may require proof of the authenticity and validity of the qualification.

6. Notwithstanding anything to the contrary in these rules contained it shall be lawful for the Council to register as a medical technologist in the category Clinical Pathology any person who, prior to the promulgation of these rules, obtained the certificate in Medical Technology of the South African Medical and Dental Council, or such other certificate or diploma recognised by the Council under the provisions of the rules promulgated under Government Notice No. 2043 of 30th September,

1949, as amended, or who obtained one of the above certificates, having commenced a recognised course of training therefor prior to the promulgation of these rules: Provided that no such certificate or diploma obtained after 31st December, 1962, shall be accepted for registration.

DEPARTEMENT VAN GESONDHEID

No. R.359]

[18 Maart, 1960.

DIE SUID-AFRIKAANSE GENEESKUNDIGE EN TANDHEEL- KUNDIGE RAAD

REÛLS VIR DIE REGISTRASIE VAN GENEESKUNDIGE TEGNOLOË

Die Minister van Gesondheid het in die uitoefening van die bevoegdheid hom verleen by subartikel (4) van artikel *vier-en-negentig* van die Wet op Geneeshere, Tandartse en Aptekers, 1928 (Wet No. 13 van 1928), soos gewysig, sy goedkeuring geheg aan die vervanging deur die volgende reëls opgestel deur die Suid-Afrikaanse Geneeskundige en Tandheelkundige Raad kragtens artikel *vier-en-negentig* gelees met artikel *twee-en-dertig* van genoemde Wet van die reëls afgekondig by Goewermentskennisgewing No. 2043 van 1949, soos gewysig by Goewermentskennisgewings Nos. 668 van 1951 en 1397 van 1954:—

1. In hierdie reëls beteken „die Wet” die Wet op Geneeshere, Tandartse en Aptekers, 1928 (Wet No. 13 van 1928), soos gewysig, en elke uitdrukking waaraan 'n betekenis by die Wet toegeskryf is, het dieselfde betekenis in hierdie reëls.

2. Die Raad kan enige persoon as 'n geneeskundige tegnoloog in een of meer van die volgende kategorieë registreer, naamlik:—

Mikrobiologie,
Chemiese Patologie,
Histopatologie,
Hematologie,
Bloedoortappingstegnologie,
Virologie,
Parisotologie en Helmintologie,
Kliniese Patologie,

wat aan die Raad bewys lewer dat hy aan die vereistes gestel in paragrawe (a), (b) en (c) van hierdie reël, voldoen het: Met dien verstande dat elke persoon wat by die afkondiging van hierdie reëls by die Raad geregistreer is as 'n geneeskundige tegnoloog kragtens die bepalings van Goewer-

mentskennisgewing No. 2043 van 30 September 1949, soos gewysig, geag word as 'n geneeskundige tegnoloog geregistreer te wees in die kategorie Kliniese Patologie:—

- (a) Dat hy in besit is van 'n goedgekeurde graad of diploma in die natuurwetenskappe van 'n erkende universiteit of van 'n eksaminerende liggaam wat van tyd tot tyd deur die Raad goedgekeur word as bevoeg om so 'n kwalifikasie toe te ken;
- (b) dat hy vir 'n tydperk van minstens vier jaar in 'n laboratorium of ander inrigting, wat deur die Raad goedgekeur is, opleiding ontvang het;
- (c) dat hy geslaag het in—
 - (i) 'n Intermediêre Eksamen wat die volgende vakke insluit:—
Chemie,
Fisika,
Anatomie,
Fisiologie,
Gebruik van Instrumente,
Algemene Laboratoriumtegniek;
 - (ii) 'n Finale Eksamen wat slegs afgelê moet word na opleiding van nie minder nie as drie jaar in 'n laboratorium of ander inrigting wat vir die doel deur die Raad goedgekeur is, in een van die volgende kategorieë:—
Mikrobiologie,
Chemiese Patologie,
Histopatologie,
Hematologie,
Bloedoortappingstechnologie,
Virologie,
Parasitologie en Helminnologie,
Kliniese Patologie:

Met dien verstande dat 'n persoon wat Kliniese Patologie beoefen, 'n bevredigende standaard in elk van die volgende vakke sal behaal:—

Mikrobiologie,
Chemiese Patologie,
Hematologie.

OPMERKINGS BY REEL 2

Opmerking (1).—Die graad of diploma wat in reël 2 (a) genoem word, moes in sy leerplan vakke ingesluit het wat wesenlik in inhoud gelykstaan met die vakke wat voorgeskryf word in reëls 2 (c) (i) en 2 (c) (ii) en die standaard van onderrig in hierdie vakke en die eksamens daarin, moet vir die Raad aanneemlik wees. Indien die Raad nie tevrede is dat die vakke of die standaard in die vakke behaal, aan die vereistes voldoen nie, kan 'n hereksamen of verdere eksamen in enige een of meer van sulke vakke geëis word.

Opmerking (2).—'n Persoon wat in besit is van 'n graad of diploma soos 'n reël 2 (a) omskryf, kan na goeddunke van die Raad vrygestel of gedeeltelik vrygestel word van die eksamens in reëls (2) (c) (i) en 2 (c) (ii) voorgeskryf; volle vrystelling van die eksamens kragtens die bepalings van hierdie opmerking sal slegs oorweeg word in die geval van 'n persoon wat vir 'n tydperk van minstens vier jaar akademiese opleiding ontvang het.

Opmerking (3).—'n Persoon wat in besit is van 'n graad of diploma soos in reël 2 (a) omskryf, kan na goeddunke van die Raad vrygestel of gedeeltelik vrygestel word van die opleiding soos omskryf in reël 2 (b); volle vrystelling van die opleiding kragtens die bepalings van hierdie opmerking sal slegs oorweeg word in die geval van 'n persoon wat akademiese opleiding vir 'n tydperk van minstens vier jaar ontvang het; 'n persoon wat in besit is van 'n goedgekeurde graad of diploma waarvan die leergang oor 'n tydperk van nie meer as drie jaar gestrek het nie, sal benewens enige eksamens wat ingevolge reël 2 (c) vereis word, na behaling van die goedgekeurde graad of diploma, ook opleiding soos voorgeskryf in reël 2 (b) vir minstens twee jaar moet ondergaan.

Opmerking (4).—'n Persoon wat in besit is van die matrikulasiesertifikaat van die Gemeenskaplike Matrikulasieraad, of 'n sertifikaat van vrystelling van die Matrikulasie-eksamen deur die Raad uitgereik, of in besit is van 'n skoolleidsertifikaat, in die eksamens waarvan die applikant in een van die vakke, Natuurkunde, Chemie, Plantkunde, Higiëne en Fisiologie, Fisika, Biologie, Dierkunde, geslaag het, of wat daarna in 'n eksamen in een van hierdie vakke geslaag het gelykstaande met of hoër as bogenoemde eksamens, en wat daarbenewens ook aan die vereistes voorgeskryf in reëls 2 (b) en 2 (c) hierbo voldoen het, kan geregistreer word.

Opmerking (5).—'n Persoon wat as geneeskundige tegnoloog in 'n laboratorium of inrigting deur die Raad goedgekeur, in enige van die kategorieë in reël 2 (c) (ii) hierbo gemeld, vir 'n tydperk van vyf jaar voor 30 September 1949 gewerk het, kan op die geskrewe aanbeveling van die hoof van daardie laboratorium of inrigting, na goeddunke van die Raad van die eksamens in reël 2 (c) voorgeskryf, vrygestel word.

Opmerking (6).—'n Persoon wat as geneeskundige tegnoloog in 'n laboratorium of inrigting deur die Raad goedgekeur, in enige van die kategorieë in reël 2 (c) (ii) hierbo gemeld, vir 'n totale tydperk van vier jaar voor afkondiging van hierdie gewysigde reëls gewerk het, kan 'n spesiale eksamen in die vak of vakke van daardie kategorie aflê, welke eksamen in die eerste plek daarop gemik is om die kandidaat se praktiese kennis van die vak wat hy beoefen, te toets, en indien hy slaag in die eksamen wat deur die Raad afgeneem moet word, kan hy geregistreer word; 'n persoon wat druipe, kan 'n verdere geleentheid gebied word om so 'n spesiale eksamen te slaag; daarna sal 'n persoon wat registrasie verlang aan al die vereistes van hierdie reëls moet voldoen; Met dien

verstande dat so 'n persoon, na goeëddunke van die Raad, van die vereistes van opleiding ingevolge reël 2 (b) hierbo, vrygestel of gedeeltelik vrygestel kan word.

Opmerking (7).—'n Persoon wat vir 'n totale tydperk van minder as vier jaar voor afkondiging van hierdie gewysigde reëls as geneeskundige tegnoloog in 'n laboratorium of inrigting deur die Raad goedgekeur, in enige van die kategorieë in reël 2 (c) (ii) hierbo gemeld, gewerk het, kan, na goeëddunke van die Raad, proporsioneel van die vereistes van opleiding ingevolge reël 2 (b) hierbo, vrygestel word.

Opmerking (8).—'n Persoon wat vir 'n totale tydperk van twee jaar of meer voor afkondiging van hierdie reëls as geneeskundige tegnoloog in 'n laboratorium of inrigting deur die Raad goedgekeur, in enige van die kategorieë in reël 2 (c) (ii) hierbo gemeld, gewerk het, kan, na goeëddunke van die Raad, vrygestel word van die vereistes om in 'n Intermediêre Eksamen soos voorgeskryf ingevolge reël 2 (c) (i) hierbo, te slaag.

Opmerking (9).—'n Persoon wat kwalifiseer vir registrasie in een of meer van die kategorieë in reël 2 (c) (ii) hierbo gemeld, kan in die register van geneeskundige tegnoloë geregistreer word, en die kategorie of kategorieë waarin hy gekwalifiseer is, moet agter sy naam op gemelde register en op die sertifikaat van kwalifikasie aan hom uitgereik, aangedui word.

3. Indien, in die geval van 'n aansoek om registrasie, die inrigting of eksaminerende liggaam op wie se sertifikaat van kwalifikasie die aansoek gebaseer is, nie reeds deur die Raad goedgekeur is nie, word van die applikant vereis om aan die Raad gesaghebbende inligting betreffende die standaard van opleiding aldaar, te verstrek, waarna, indien die standaard van opleiding deur die Raad bevredigend geag word, word dié inrigting of eksaminerende liggaam goedgekeur.

4. 'n Applikant om registrasie ooreenkomstig hierdie reëls moet die bewys en kwalifikasies op grond waarvan hy aanspraak maak om registrasie, tesame met die volgende voorlê—

- (a) 'n verklaring van identiteit wat voor 'n vrederegter of kommissaris van ede beëdig is;
- (b) 'n sertifikaat van goeie karakter onderteken deur 'n geregistreerde persoon, 'n predikant, 'n landdros of 'n ander verantwoordelike persoon;
- (c) 'n sertifikaat van 'n geregistreerde geneesheer dat die gesondheid van die applikant nie sodanig is dat dit onraadsaam is dat die applikant sy beroep beoefen nie;
- (d) 'n beëdigde verklaring voor 'n vrederegter of kommissaris van ede deur die applikant afgelê dat hy nooit in enige land as gevolg van 'n misdryf of professionele wangedrag verbied is om te praktiseer nie;
- (e) 'n bedrag van £5 vir registrasie;

(f) 'n geboortesertifikaat, of, indien die applikant nie in staat is om sy geboortesertifikaat te verstrek nie, 'n doopseël of ander bevredigende bewys dat hy die ouderdom van een-en-twintig jaar bereik het.

5. Die Raad kan eis dat bewys gelewer word van die egtheid en geldigheid van die kwalifikasie.

6. Ondanks andersluidende bepalings in hierdie reëls, is die Raad regtens bevoeg om enige persoon as 'n geneeskundige tegnoloog in die kategorie Kliniese Patologie te registreer wat voor die afkondiging van hierdie reëls die sertifikaat in Geneeskundige Tegnologie van die Suid-Afrikaanse Geneeskundige en Tandheelkundige Raad behaal het, of sodanige ander sertifikaat of diploma deur die Raad erken kragtens die bepalings van die reëls afgekondig by Goewermentskennisgewing No. 2043 van 30 September 1949, soos gewysig, of wat een van bogenoemde sertifikate behaal het nadat 'n aanvang met 'n erkende opleidingskursus daarvoor gemaak is voor die afkondiging van hierdie reëls: Met dien verstande dat geen sodanige sertifikaat of diploma wat na 31 Desember 1962 behaal is, vir registrasie aangeneem word nie.

ABSTRACTS

HISTOPATHOLOGY *submitted by* A. Scott.

BIOCHEMISTRY, MICROBIOLOGY *submitted by* N. Richardson.

A simple apparatus for embedding small Histological specimens in paraffin.

A paraffin embedding bath, easily made from copper tubing, copper plate and a warming plate for embedding small specimens is described. Seno, T. (1959). *Stain Tech.*, **34**, 157.—V.J.E.

A new technique for the Histochemical study of smears.

To overcome the difficulty of smears floating off glass slides a polyester plastic film was used instead and the usual dehydrating and clearing techniques could still be used. Burstone, M. S. and Flemming, T. J. (1959). *J. Histochem. Cytochem.*, **7**, 203.

Histochemical demonstration of copper in copper-fed rats and in Hepatolenticular degeneration.—Four histochemical methods for the demonstration of copper were compared; sodium diethyl dithiocarbamate, rubeanic acid, p-dimethylaminobenzylidene rhodamine and sym-diphenylcarbazine methods. The sodium diethyldithiocarbamate method gave the most consistent and sensitive results. Howell, J. S. (1959). *J. Path. Bact.*, **77**, 473.

Staining of fresh undecalcified thin bone sections.

These can be reproducibly and reliably stained by any of the following procedures:—

(a) Basic fuchsin, 1 per cent. in 30 per cent. alcohol for 48 hours at 22°C.

(b) Silver nitrate, 0.33M, for 48 hours at 22°C. washing for 48 hours in a large volume of distilled water; exposure to light to develop the colour.

(c) Metallic sulphides: the nitrate of metal, 0.33M for 48 hours at 22°C., then Na₂S, 0.33M for 48 hours at 22°C.

(d) Alizarin red S, 0.1 per cent. for 48 hours at 22°C., differentiated for 48 hours at 22°C. in weakly alkaline water, pH about 8.

(e) KMnO₄: boiling 8—10 mins. in a 0.1 N solution.

With the exception of (c) the surface stain must be ground off the section for microscopic examination of its interior. Stain concentration, time and temperature can be altered to suit specific needs. Frost, H. M. (1959) *Stain Tech.*, **34**, 135.

The removal of mercury after fixation in sublimate-containing mixtures.

The author notes that if mercury-fixed tissues are dehydrated in cellosolve, removal of mercury pigment is unnecessary. Gonzalez, R. (1959) *Stain Tech.*, **34**, 111.

A simple automatic Ultramicrotome.

Details are given for making this instrument for electron microscopy. The forward motion of the specimen is effected by an electrically heated resistance wire and the up and down motion across the knife by gravity and air pressure. Buck, R. C. and Jarvis, C. E. (1959) *Stain Tech.*, **34**, 109.

Dimedone as an aldehyde blocking reagent to facilitate the histochemical demonstration of glycogen.—By treating the sections with 5 per cent. alcoholic dimedone at 60°C. for 3 hours following periodic acid oxidation, the PAS positive reaction of the majority of substances other than glycogen is blocked. Bulmer, D. (1959) *Stain Tech.*, **34**, 95.

BOOK REVIEWS

Tools of Biological Research. 183 pages. Editor: Hadley J. B. Atkins. Blackwell Scientific Productions 1959. 37s. 6d.

This symposium has been compiled to provide research workers with a guide to instruments and methods used in modern laboratories.

The text deals with both physical and practical aspects of the methods described, in a highly interesting manner.

The chapters on Flame photometry, Electrophoresis, the Electron Microscope and Tissue Culture should prove to be of especial interest to all students of Medical Technology.

This book should have a place in all medical laboratory libraries.

E.J.W.

Oxosteroids—the use of hydrazides for detection, characterisation and estimation. Bernard Camber, M.D. Pp. vii + 79. London: H. K. Lewis & Co. Ltd. 1960.

This book is based on a thesis for a degree of Doctor of Medicine and the object was an attempt to find improved reagents and methods for the recognition and estimation of steroid compounds with hormonal activity in body tissues and fluids for, as the author clearly expresses, there is ample room for a "sabbatical year" devoted to improved methodology in steroid analysis in relation to body fluids and tissues.

By and large this book is of interest to the steroid chemist. The author systematically develops his fundamental approach based on hydrazone formation. There is a preliminary investigation of suitable hydrazides, followed by a fuller investigation of the chemical behaviour, suitability and application. The histochemical application was briefly investigated in some instances.

This work bears ample testimony to original thinking and systematic application, and anybody familiar with steroid analysis will readily appreciate the sheer hard work the investigation involved. Without doubt, those interested in analytical methodology in steroid chemistry will find much of interest. The practicability of much of the work presented, however, is as yet undecided.

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The Director of the Amoebiasis Research Unit, C.S.I.R./University of Natal/Natal Provincial Administration announces that a competition will be held during 1960 similar to that recently conducted by this Journal. Conditions of entry are as follows:—

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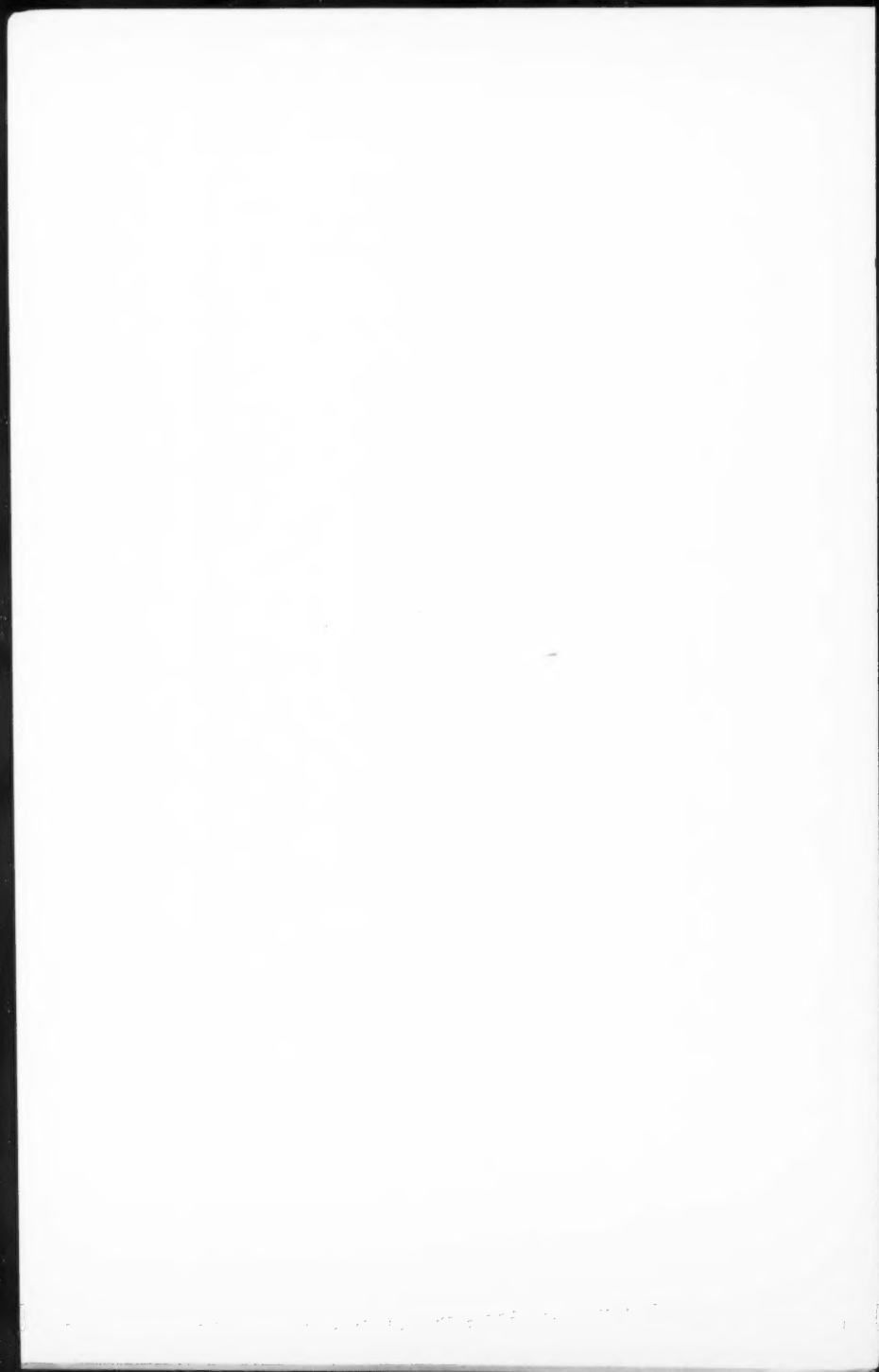
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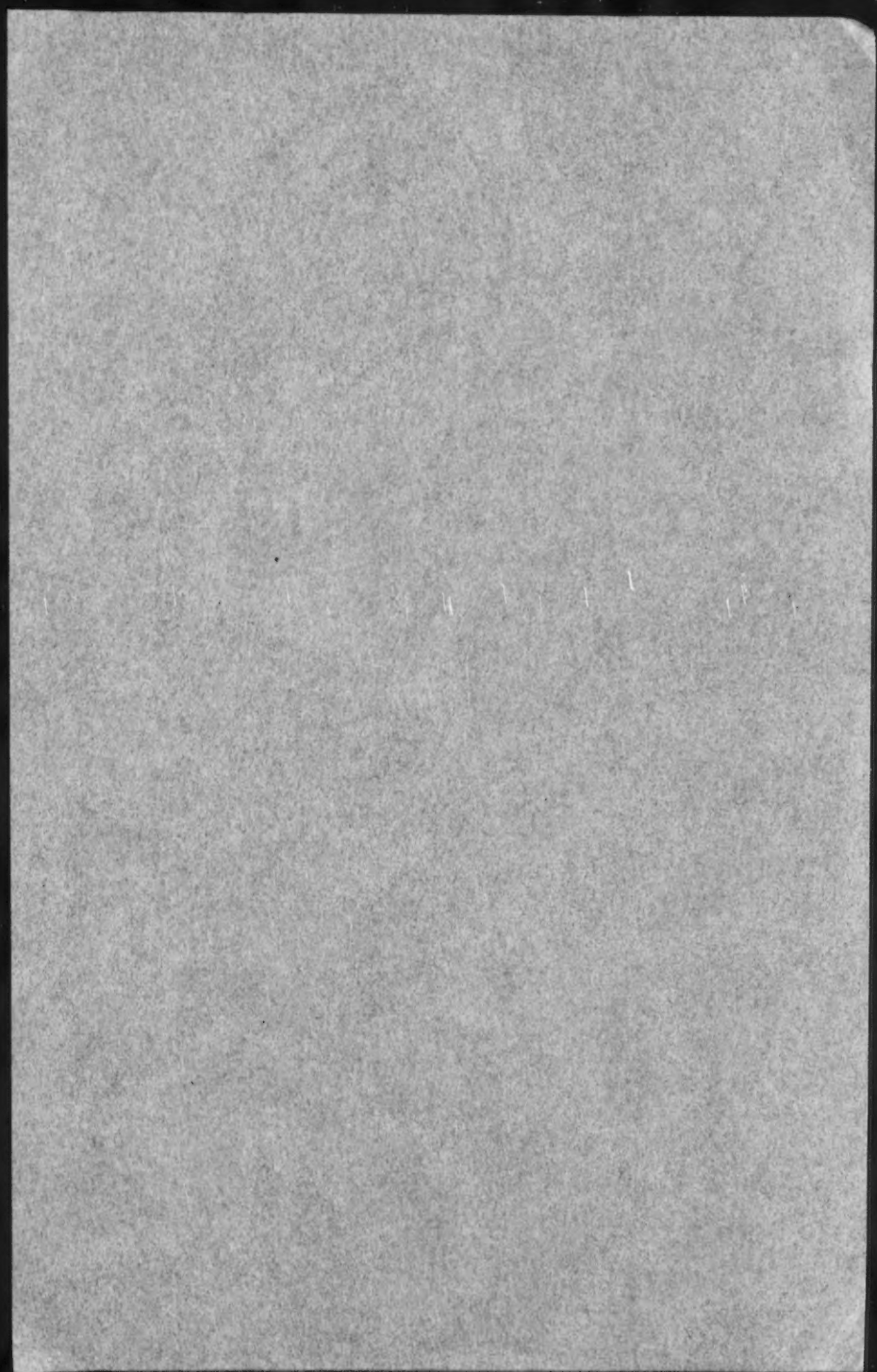
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